Application Serial No.: 10/009,315

Group Art Unit No.: 1634

## **Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

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## **Listing of Claims:**

Claims 1-27. (Cancelled)

- 28. (Currently Amended) A method for inserting a cassette into a DNA molecule to produce a DNA sequence fusion cassette without requiring ligation, said method comprising the steps of:
- (a) providing a selected DNA molecule comprising a first region of DNA sequences upstream of a site targeted for disruption and a second region of DNA sequences downstream of the site targeted for disruption, wherein said first region of DNA sequences upstream of a site targeted for disruption further comprises a first strand having a first and a second end, and said second region of DNA sequences downstream of the site targeted for disruption further comprises a first strand having a first and a second end;
- (b) providing a cassette comprising a first strand of DNA, wherein the first strand comprises at its 5'end DNA sequences which overlap with sequences at the second end of the first region of DNA sequences upstream of a site targeted for disruption, and at its 3' end DNA sequences which overlap with sequences of the first end of the second region of DNA sequences downstream of the site targeted for disruption;
- (c) amplifying the first region of DNA sequences upstream of a site targeted for disruption using primers for said first region and amplifying the second region of DNA sequences downstream of the site targeted for disruption using primers for said second region;
  - (d) mixing the cassette with the amplified first and second regions;
- (e) amplifying the mixture of (d) using polymerase chain reaction, thereby producing without ligation a DNA sequence fusion cassette comprising the first region of DNA sequences and second region of DNA sequence flanking the cassette, wherein amplifying further comprises the steps of: The method according to claim 24, wherein the amplifying step (e) further comprises the steps of:
- (i) heating the mixture of (d) for about 5 minutes in the absence of polymerase or primers at about 94[[EC]]°C;
  - (ii) cooling the heated mixture of (i) to 50[[EC]]°C over about 30 minutes;

- (iii) maintaining the mixture at about 50[[EC]]°C for about 5 minutes;
- (iv) adding a thermostable polymerase to the mixture;
- (v) adding a proof-reading polymerase with 3' exonuclease activity to the mixture;
  - (vi) heating the mixture to about 72[[EC]]°C for about 5 minutes; and
- (vii) adding to the mixture primers comprising a 5' forward primer P1 and a 3' reverse primer P4 for the nucleotide sequence region downstream of the target site.
- 29. (Currently Amended) A method for inserting a cassette into a DNA molecule to produce a <u>DNA sequence fusion cassette</u> nucleic acid cassette fusion without requiring ligation, said method comprising the steps of:
- (a) providing a first region of DNA sequences and a second region of DNA sequences, said first and second regions each comprising a first strand having a first and second end;
- (b) mixing with the first and second regions a cassette comprising a first strand of DNA, wherein the first strand comprises at its 5' end DNA sequences which overlap with sequences at the second end of the first region, and at its 3' end DNA sequences which overlap with sequences of the first end of the second region;
  - (c) mixing the cassette with the first and second regions;
- $(\underline{c})[[(d)]]$  heating the mixture of  $(\underline{b})[[(c)]]$  for about 5 minutes in the absence of polymerase or primers at about  $94[[EC]]^{\circ}C$ ;
- (d)[[(e)]] cooling the heated mixture of (c) to 50°C (i) to 50EC over about 30 minutes;
- (e)[[(f)]] maintaining the mixture at about 50[[EC]]°C for about 5 minutes;
  - (f)[[(g)]] adding a thermostable polymerase to the mixture;
- (g)[[(h)]] adding a proof-reading polymerase with 3' exonuclease activity to the mixture;
  - (h)[[(i)]] heating the mixture to about  $72[[EC]]^{\circ}C$  for about 5 minutes;
  - (i)[(j)]] adding to the mixture primers comprising a 5' forward primer

P1 for the first region and a 3' reverse primer P4 for the second region, and

(j)[[(k)]] amplifying the mixture of (i)[[(j)]] using polymerase chain reaction, thereby producing without ligation a DNA sequence fusion cassette comprising the

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first <u>region of DNA sequence</u> and second <u>region of DNA sequence</u> flanking the cassette;

Claims 30-32 (Cancelled)